# Substitute Specification – Clean Version

### **Claims**

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## What is claimed is:

1. A biosensor for determination of an analyte concentration in a test sample comprising:

a mixture for electrochemical reaction with an analyte, said mixture including an enzyme,

a mediator, and an oxidizable species as an internal reference.

- 2. The biosensor of claim 1 wherein said internal reference is a reduced form of a reversible redox couple that has an equal or higher redox potential than that of said mediator.
  - 3. The biosensor of claim 1 wherein said mediator comprises 3-phenylimino-3H-phenothiazine.
  - 4. The biosensor of claim 3 wherein said internal reference comprises ferrocyanide.
  - 5. The biosensor of claim 4 wherein said ferrocyanide and said mediator are oxidized at a first voltage potential and only said mediator is oxidized at a second voltage potential[[;]], said second voltage potential being less than said first voltage potential.
- 6. The biosensor of claim 5 wherein said first voltage potential is about 400 mV and said second voltage potential is about 100 mV.
  - 7. The biosensor of claim 1 wherein said mediator comprises ferricyanide.
  - 8. The biosensor of claim 7 wherein said internal reference comprises ferrocyanide.

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- 9. The biosensor of claim 1 wherein said mediator comprises ruthenium hexaamine.
- 10. The biosensor of claim 9 wherein said internal reference comprises ferrocyanide.
- 11. The biosensor of claim 10 wherein said enzyme comprises glucose oxidase.
- 12. A method of use of a biosensor including a mixture of an enzyme, a mediator, and an oxidizable species as an internal reference, said method comprising the acts of:

applying a first voltage potential in a first period; and providing a set delay period;

applying a second voltage potential in a final period following said delay period, and wherein said first voltage potential and said second voltage potential are selectively provided for oxidizing only said mediator or both said mediator and said internal reference.

- 13. The method of claim 12 wherein the act of applying said first voltage potential in said first period includes the act of applying a selected high first voltage potential in the first period for oxidizing said mediator and said internal reference.
- 14. The method of claim 12 wherein the act of applying said first voltage potential in said first period includes the act of applying a selected low first voltage potential in the first period for oxidizing only said mediator.
- 15. The method of claim 12 wherein the act of applying said second voltage potential in said final period following said delay period includes the act of applying a selected second voltage potential for oxidizing said mediator and said internal reference.

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- 16. The method of claim 12 wherein the act of applying said second voltage potential in said final period following said delay period includes the act of applying a selected second voltage potential for oxidizing only said mediator.
- 17. The method of claim 12 wherein the acts of applying said first voltage potential and applying said second voltage potential includes the acts of applying a selected voltage potential in a range between 100 mV and 400 mV.
- 18. The method of claim 12 wherein the acts of applying said first voltage potential and applying said second voltage potential includes the acts of applying a selected first voltage potential in the first period for oxidizing both said mediator and said internal reference; and applying a selected second voltage potential for oxidizing only said mediator.
- 19. The method of claim 12 wherein the biosensor includes a mediator comprising one of 3-phenylimino-3H-phenothiazine and ruthenium hexaamine; and wherein the internal reference comprises ferrocyanide; and wherein the acts of applying said first voltage potential and applying said second voltage potential includes the acts of applying a selected first and second voltage potential for oxidizing only said mediator.
- 20. The method of claim 12 wherein the acts of applying said first voltage potential and applying said second voltage potential includes the acts of applying a selected first and second voltage potential for oxidizing both said mediator and said internal reference; wherein said internal reference effectively anchoring a calibration intercept within a narrow range and said internal reference effectively maintaining a calibration slope for the biosensor.

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